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NOTES ON EXPERIENCES WITH THE PRESUMPTIVE AND CONFIRMATORY TESTS FOR B. COLI IN WATER ANALYSIS FOR THE AMERICAN EXPEDITIONARY FORCES[1]

By Max Levine[2]

In the routine bacteriological analysis of water, disease producing microörganisms are not sought for nor are they likely to be detected, even in a dangerous water. It devolves upon the analyst to interpret his bacteriologic findings, and in this he is aided materially by the determination of the incidence of B. coli. Tests for this organism were carried out in the following manner:

Five 10 cc., two 1 cc. and one 0.1 cc. portions of the water under consideration were placed in lactose broth and incubated at 37°C. After twenty-four hours, the amount of gas in each tube was recorded and those showing no gas were replaced in the incubator for another twenty-four hours. Gas formation in one or two days was regarded as presumptive evidence that B. coli may be present, but to confirm this presumption, such tubes were streaked on eosine-methylene-blue agar to be described below.

THE PRESUMPTIVE TEST FOR B. COLI

In dealing with untreated waters, the production of 10 per cent or more gas after twenty-four hours is recognized as a very reliable index of the probable presence of B. coli or its close allies. This is a positive presumptive test. A slower rate of gas production (i.e., where there is less than 10 per cent gas in twenty-four hours, or more than this quantity in forty-eight hours where twenty-four hours was negative) is regarded as an inconclusive or doubtful presumptive test and further confirmation must be carried out.

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[1] Read before the Iowa Section, October 22, 1919. Authorized for publication by the Surgeon-General, U. S. Army. Discussions of this paper are requested and should be sent to the Editor.

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These limitations of the reliability and applicability of the presumptive test were not properly appreciated by many likely to be called upon to examine and report on water specimens. It was the rather prevalent opinion that a small amount of gas in twenty-four hours was of little significance, whereas the production of 10 per cent or more gas in forty-eight hours was regarded as a good index of the probable presence of B. coli. As the presumptive test is often the only one available under campaign or field conditions, and indeed, is too frequently relied upon in the control of water purification plants, it was felt that the observations on this reaction at the Central Medical Department Laboratory of the American Expeditionary Forces, based upon the examination of 1559 specimens and comprising waters from both treated and raw supplies from various sources, would be of interest, and perhaps of value for record.

TABLE 1

Showing correlation of rate of gas production with confirmation of the presumptive test for B. coli in lactose broth

RATE OF GAS PRODUCTION	UNTREATED SUPPLIES		CHLORINATED SUPPLIES	
	Number of tubes showing gas	Percentage of gas tubes confirmed	Number of tubes showing gas	Percentage of gas tubes confirmed
Rapid (10 per cent or more in twenty-four hours)	684	97.7	57	44.0
Moderate (less than 10 per cent in twenty-four hours)	193	91.2	15	20.0
Slow (no gas in 24 hours; 10 per cent or more in forty-eight hours)	276	73.2	156	6.4
Very slow (no gas in twenty-four hours; less than 10 per cent in forty-eight hours.)	33	45.5	26	0.0

From table 1, it is evident that

1. *The positive presumptive test* (10 per cent or more gas in twenty-four hours) is a very reliable index of the probable presence of B. coli when dealing with untreated waters but it is not to be depended upon when testing chlorinated waters; 97.7 per cent of the former and only 44 per cent of the chlorinated samples showing gas, were confirmed for B. coli or its close allies.

2. *The doubtful presumptive test* (less than 10 per cent gas in twenty-four hours, or more than this quantity in forty-eight hours

where twenty-four hours was negative) is only a fair index of the probable presence of B. coli in untreated waters, while for chlorinated specimens it is practically negligible, as 75.9 per cent of gas tubes from untreated, and only 7.6 per cent of those from chlorinated samples were successfully confirmed.

3. A small amount of gas in twenty-four hours is a more reliable index for B. coli than 10 per cent or more gas in forty-eight hours. The rate of gas production seems more significant than the total volume of gas formed as a presumptive test.

THE PARTIALLY CONFIRMED TEST

For confirming the presumptive test, the simplified eosine-methylene-blue agar was employed in place of the Endo medium because of its ease of preparation and greater stability. This medium also enables us to differentiate between the B. coli and B. aerogenes in a large proportion of instances simultaneously with confirming the presumptive test, as indicated in table 2.

TABLE 2

Reliability of presumptive differentiation of B. coli and B. aerogenes on Eosine-Methylene Blue agar

DESIGNATION FROM APPEARANCE ON AGAR	1918–1919, FRANCE		1917, IOWA	
	Nunber of colonies tested	Percentage correct	Number of colonies tested	Percentage correct
B. coli....................	87	94.2	122	96.9
B. aerogenes.............	55	85.5	102	82.4

Preparation of simplified eosine-methylene-blue agar. This is made up of distilled water, 1000 cc.; peptone (Difco), 10 grams; dipotassium phosphate (K_2HPO_4), 2 grams; agar, 15 grams.

Boil ingredients until dissolved, and make up any loss due to evaporation with distilled water.

Place measured quantities in flasks or bottles and sterilize in the autoclave at 15 pounds pressure for fifteen to twenty minutes.

Just prior to use add to each 100 cc. of the melted agar prepared as above the following: Lactose, 1 gram or 5 cc. of 20 per cent solution; eosine (2 per cent yellow aqueous solution), 2 cc.; methylene blue (0.5 per cent aqueous solution), 2 cc.

Pour medium into petri dishes, allow them to harden, and inoculate by streaking on the surface.

There is no adjustment of the reaction, and filtration of the medium is not necessary.

PRESUMPTIVE DIFFERENTIATION OF B. COLI AND B. AEROGENES ON EOSINE-METHYLENE-BLUE AGAR

B. coli forms flat, dark-centered, button-like colonies 2 to 4 mm. in diameter, which by reflecting light show a characteristic greenish metallic sheen. The colonies of B. aerogenes are generally much larger, convex, with brownish centers, and rarely exhibit a metallic lustre.

To determine the reliability of the presumptive differentiation of B. coli and B. aerogenes on the eosine-methylene-blue agar, 153 colonies were fished from routine confirmatory plates, designated as B. coli or B. aerogenes and later identified more completely. Of 87 supposedly B. coli 94.2 per cent were confirmed, while of 55 designated as B. aerogenes 85.5 per cent were such. Of 11 strains recorded as questionable but probably B. aerogenes, 72.7 were correctly recorded. As this differentiation is obtained simultaneously with the confirmatory test, requiring no additional labor, the use of the eosine-methylene-blue agar should prove of considerable value for routine water analysis.

The presumptive and confirmatory tests are designed to detect all members of the colon group of bacteria. In examining waters we are interested particularly in determining whether wastes from human sources are present, and B. coli is employed as the index. It should be borne in mind, however, that the habitat of coli-like bacteria is not restricted to the human intestine. Similar organisms are characteristic of the animal intestine, and not infrequently are found in the soil and upon grains.

The desirability of differentiating coli-like bacteria from different sources has long been recognized, for it is evident that human pollution is much more dangerous to the public health than animal wastes, and that the latter, in turn, are considerably more objectionable than contamination with soil washings. It is at present impossible to distinguish B. coli from human and other animal sources, but much evidence has been accumulated which indicates that the predominating animal forms are quite distinct from the prevailing types obtained from the soil and upon grains.

Recent studies have demonstrated conclusively that whereas B. coli is a constant inhabitant of the intestinal tract of man and other animals, it is comparatively infrequently encountered in soils, and very rarely found upon grains. B. aerogenes and B. cloacae, on the other hand, are rare in feces, predominate in the soil, and are practically the only coli-like forms obtained from grains.

The comparative viability of B. coli and B. aerogenes in water, and their relative resistance to chemical or other treatment should be considered in interpreting water analysis. B. aerogenes is said to be more viable and more resistant to treatment; it is even said to multiply in natural waters. In view of these facts, if they are substantiated by further studies and observations, the same sanitary significance should not be accorded B. aerogenes as is given to findings of B. coli, particularly when considering stored or chlorinated waters.

The raw water of the Dijon, France, water supply, examined daily for six months, yielded 96 positive results for members of the colon group, with B. aerogenes present in 15.6 per cent of these. The tap samples during the same period (water was treated with 0.07 to 0.1 part per million chlorine) only showed three positively confirmed specimens while the plant was in operation, but B. aerogenes was present in each instance or 100 per cent of positively confirmed gas tubes. The indication is therefore strong that the B. aerogenes is more resistant to chlorination than is B. coli.

It is not feasible, from our present incomplete knowledge of the types, to put forth any set rules for interpreting the significance of the presence of various members of the colon-aerogenes group in water, but the following is offered as a suggestion.

In ground waters, the presence of B. aerogenes may be given the same weight in the interpretation as is accorded to the presence of B. coli, on the assumption that such waters are essentially filtered supplies and should be free from soil washings. If soil forms of coli-like bacteria are present it may be regarded as an indication of imperfect filtration or improper surface protection, either condition making the supply suspicious.

In stored surface or treated waters, the presence of B. aerogenes alone, on account of its more probable non-fecal origin, greater longevity in water, and apparently greater resistance to treatment, should not be regarded as objectionable as is the presence of the true B. coli in equal numbers.

The proper differentiation of B. coli from B. aerogenes may thus be of practical importance to those who have to deal with treated or surface supplies.